

Testing for Bovine Tuberculosis and Chronic Wasting Disease in Michigan's Wildlife

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Wildlife personnel with the Michigan Department of Natural Resources (MDNR) have been examining harvested deer submitted by hunters for over 50 years. This activity has been an important source of biological data on the status and health of the deer herd. With the discovery of bovine tuberculosis (TB) in wild deer, a disease surveillance and monitoring program was necessary. In 2002, due to the detection of Chronic Wasting Disease (CWD) in Wisconsin, a surveillance program for CWD in Michigan was initiated. The voluntary check of deer by hunters has facilitated these efforts.

The process starts with hunters voluntarily examining their deer's ribcage and lungs, and submitting heads and suspicious tissues to MDNR check stations. Field check stations are located throughout the state, mostly at MDNR offices. During rifle deer season in November there are additional check stations on southbound lanes of major highways, at the Mackinac Bridge, and at other field locations convenient to the hunter. Cooperation from owners of meat processing plants, taxidermy shops, and other businesses that cater to deer hunters has also had a positive impact on the disease surveillance effort.



Deer Check Station at Mackinac Bridge

At a check station, hunters are asked several questions about the date and location of kill and check station personnel record biological data. Starting in 2001, check station staff began using hand-held computers called Personal Digital Assistants, or PDAs, to record the biological data. Using a custom application designed by the MDNR, staff is able to electronically record all information that was previously recorded by hand. The age of the deer is determined by examining tooth replacement and wear patterns. Antler development is measured on all adult bucks by taking beam diameters and counting the number of points. The presence or absence of lactation is noted for all does. A visual check of the chest cavity for the presence of abnormal tissues is made. Unusual coat color or other diseases and anomalies are noted. Hunters are offered a Management Cooperators patch for any deer brought to these check stations.



Beam diameter taken at a deer check station.

Depending on where the deer was harvested, hunters are asked if they want to submit the deer head for either TB or CWD testing. If agreed,

check station personnel will cut off the head an inch or so behind the ears, remove antlers, if desired by the hunter, and attach an appropriate disease tag. Beginning in 2002, bar code scanners were added to the PDAs, a barcode tag number was added to the disease tags and a barcode driver's license or sport card number was added to the deer kill tags. This information can be scanned into the PDAs and later uploaded into a computer database. The number scanned from the kill tag can later be used to automatically look up the hunter's name and address. The hunter receives the tear off portion on the disease tag with an identification number and will be contacted after the test results are completed. If a hunter chooses to leave a deer carcass intact for any reason, disease tags are completed at the check stations but given to the hunter to turn in with the deer head at a later date. Skinned and de-antlered skulls may still be examined for bovine TB and CWD. Hunters are also requested to take entire deer carcasses to check stations if they see abnormal tissues in the lungs or on the ribs. Hunters are given a replacement deer license at the check station if MDNR personnel determine that the abnormal tissues look typical of tuberculosis. Also, there is a mandatory check for all hunter harvested and road-killed elk. Every elk head is submitted for TB and CWD testing and follows the same procedures as the deer heads.



Inside the BL3 necropsy

After the heads are collected at check stations they are brought to the MDNR's Wildlife Disease Laboratory (WDL). In early fall 2004, the WDL moved from its former location at the Rose Lake Wildlife Research Center to the new Diagnostic Center for Population and Animal Health (DCPAH). This facility is shared with Michigan State University's (MSU) Diagnostic Lab and is located south of MSU's main campus.

Inside DCPAH is a Biosecurity Level 3 (BL3) necropsy where the heads are processed. This BL3 necropsy room is used to contain any potentially infectious materials and workers wear full personal protection equipment and are required to shower out of the facility.

Heads are delivered throughout the year, but during the peak time, from mid-November through early-December, several truckloads arrive daily. Some days the number of heads received can be as much as a couple thousand!

The heads are unloaded from trucks and placed in carts to be taken into the BL3 necropsy. Here they are removed from bags and are re-examined to make sure the age and sex has been recorded on the disease tag that is attached. Once examined, part of the numbered tag is clipped from the head, leaving an identical number on the portion of the tag still attached to the head. Heads are separated and handled in different ways depending on whether they are being tested for TB or CWD.

Heads that will only be examined for TB are taken to tables where personnel can examine the lymph nodes. Each head is visually (grossly) examined. Specific lymph nodes sectioned for inspection in each head include lymph nodes near the temple (**parotid**), behind the lower jaw (**submandibular**), and the lymph nodes at the base of jaw near the vertebrae (**medial retropharyngeal**).



Examining lymph nodes in a deer head.

Deer heads in which **abscesses (gross lesions or puss within one or more lymph nodes)** are not found are determined to be **negative** for



Tuberculosis abscess in retropharyngeal lymph node.



Deer lungs and ribs showing extensive infection.

bovine TB and no further TB testing is done. Whole deer carcasses and/or abnormal tissues that are submitted are examined by one of the veterinarians or the pathologist who makes notes about lesions found in the deer.

If gross lesions suggestive of bovine TB are found in any of the tissues examined, the deer is identified as a **suspect**. Abscessed lymph nodes from a suspect deer are divided into two containers. One container is sent to Michigan Department of Community Health (MDCH). The other is sent to MSU's Diagnostic Lab and examined for histopathological (the study of microscopic changes in diseased tissues) lesions that are typical of disease caused by the bovine TB bacteria, *Mycobacterium bovis* (*M. bovis*). The histologic lesion most commonly associated with bovine TB is called a **granuloma**. It is a thick-walled capsule (a **tubercle or nodule**) that forms around the bacteria. In addition to looking for specific lesions under the microscope, pathologists at MSU's Diagnostic Lab and at MDCH use a special stain to microscopically identify bacteria that belong to the *Mycobacterium* family and could be the bovine TB bacteria. This is called an **acid-fast stain test**. The cell walls of bacteria belonging to the *Mycobacterium* family will take up a stain that can't be rinsed off and will appear as short red or pink rods under a microscope.

The finding of acid-fast-staining organisms in tissues is only suggestive of infection with *M. bovis* - other bacteria may also retain the stain. More definitive laboratory testing is required to make the diagnosis of *M. bovis* infection. The final testing is done at MDCH where they attempt to **culture** (grow) the *Mycobacterium bovis* bacteria. This bacteria is very slow-growing and can take 8-16 weeks to grow. Therefore, a culture is not called negative for bovine TB until after it has been incubated for 3-4 months. If the culture does grow, it is subject to further testing to determine if the isolate is *Mycobacterium bovis* or some other closely related *Mycobacterium* species. Each species of *Mycobacterium* has specific biochemical characteristics and genetic makeup that leads to a **final identification** of the bacteria.

Deer heads submitted for CWD testing are also examined visually for TB. One of the medial retropharyngeal lymph nodes located at the base of the jaw near the vertebrae is removed and sectioned with one small piece, approximately 200 mg cut into 3-4 pieces, placed in a small grinding tube filled with a buffer solution. The rest of the lymph node is placed into a whirl-pak bag and frozen to keep as a back up sample. The pieces of lymph



Lymph nodes are sectioned and weighed for CWD testing.

node in the grinding tube are sent on to MSU's Diagnostic Lab and placed in a homogenizer to grind up the tissue. An ELISA test is then performed to detect any positive CWD cases. To date there have been no positive CWD cases in Michigan. After all heads have been processed, all exposed surfaces in the laboratory are disinfected with a tuberculocide. All deer and elk heads and parts are placed in a lined cart for incineration.

The disease tags that have been clipped are put into a disinfectant bath and soaked for at least 15 minutes. They are then sent through a dip tank pass through into our Biosecurity Level 2 (BL2) necropsy. Here they are rinsed and dried, which prepares them to be handled by personnel in order for the information to be entered into a database. Once the tag number is entered into the database for tags that have been previously scanned into the PDA's, the hunter and location information associated with that tag will automatically be uploaded from the main database. This results in less information having to be key punched and a big reduction in data entry time. Information that is not scanned has been written on the tag and is key-punched manually into the database. The addresses that have been collected either by writing them on the tag or linking them with the kill tag number are used to send hunters results of their TB or CWD test. Hunters can also use the disease tag number they are given at the check station or their driver license or sportscard number to check their results on-line at <http://secure1.state.mi.us/testresults>

The location where the deer was taken is important in order to keep track of how many deer have been tested in each county and also to locate exactly where the TB positive deer have come from. This information is displayed on maps that are handed out at meetings and to the public. Most of the maps are created at the MDNR's WDL, including the TB positive deer map, non-cervid TB tested map, the all animals TB positive map and CWD testing maps. These maps and summary tables are posted on the website at <http://www.michigan.gov/bovinetb>

The Bovine TB Eradication Project member agencies, MDNR, MDCH, the Michigan Department of Agriculture, MSU, and the United States Department of Agriculture, have been working cooperatively on bovine tuberculosis surveillance and eradication since 1994 when the first TB positive deer was found. The Project involves an outstanding effort and long hours by all people involved. To date over 138,567 deer have been tested for TB since 1994, and 17,583 deer tested for CWD since 2002.

**2005 Time-Line for hunter notification of lab test results.
Questions: Contact Jean at (517) 336-5033 or fierkej@michigan.gov**

Type of sample submitted & tests requested:	If determined by Wildlife Disease Lab to be negative for bovine TB and/or CWD:	If determined by the Wildlife Disease Lab to be a suspect for bovine TB or the deer is a part of the TB Blood Study:
Deer head for TB - White tag Deer head for TB Blood Study - Blue tag	Hunter will receive a postcard with bovine TB results within 10 days from when the sample is received at the Lab. Deer heads that are a part of the TB Blood Study will not receive a postcard.	Hunter will receive the first letter within 14 days from when the sample was received at the Lab, stating that the sample is a suspect for bovine TB or stating that it is a part of the TB Blood Study. These samples will undergo bacterial culture. A letter with the final results (negative or positive), for suspects and TB Blood Study heads, will be mailed within 2-3 months of when the sample was received at the Lab.
Deer or Elk head - Orange tag for CWD & TB	Hunter will receive a postcard with CWD results within 20 days from when the sample is received at the Lab. Hunter will also receive bovine TB result as shown above (will receive separate postcards for CWD and TB if both tests are negative).	If TB abscesses are found, the hunter will be notified as shown above. If the CWD test is suspect, the hunter will be called on the phone by the Lab (it is very unlikely there will be any CWD suspects).
Suspicious tissues for TB & CWD with or without a head - Yellow tag (carcass, lungs, misc. tissue with abscess)	Hunter will receive a laboratory report with TB results and diagnosis within 2 weeks to a month from when the sample is received at the Lab. Cards will not be sent.	Hunter will receive the first letter within 14 days from when the sample was received at the Lab, stating that the sample is a suspect for bovine TB. A letter with the final results (negative or positive) and a laboratory report will be mailed within 2-3 months of when the sample was received at the Lab.
Negative TB results for deer heads will be available on the web the day after they are examined by the Lab. Negative CWD results for deer heads will be available on the web about 10 days after being examined by the Lab.		
https://secure1.state.mi.us/testresults/		